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Diversity-Oriented Synthesis of Azapeptides with Basic Amino Acid Residues: Aza-Lysine, Aza-Ornithine, and Aza-Arginine

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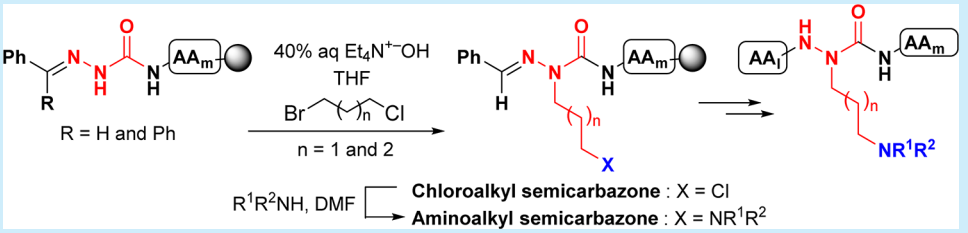
Supporting Information



ABSTRACT: Aza-peptides with basic amino acid residues (lysine, ornithine, arginine) and derivatives were synthesized by an effective approach featuring alkylation of a hydrazone-protected aza-glycine residue with α-bromo ω-chloro propane and butane to provide the corresponding alkyl chloride side chains. Displacement of the chloride with azide and various amines gave entry to azaOrn, azaLys, and azaArg containing peptides as demonstrated by the solution and solid-phase syntheses of 29 examples, including an aza-library of Growth Hormone Releasing Peptide-6 analogs.

Basic amino acid residues, such as lysine and arginine, function in numerous biological processes including post-translational modifications,[1] transport across membranes,[2] and as enzymatic cleavage sites.[3] For example, acylation and methylation of lysine residues of histone proteins alter chromatin structure and gene regulation.[1,4] Cell-penetrating peptides,[5,6] such as Tat and penetratin, are composed of multiple basic residues. The protease trypsin cleaves peptides specifically at the *C*-terminal of lysine and arginine residues.[7] Peptide mimics with constrained conformations and chemical modifications at lysine and arginine residues are thus desirable tools for studying and regulating such phenomena.[8−14]

Azapeptides employ the electronic constraints of a semicarbazide residue to rigidify peptide geometry in favor of turn conformations, with the added benefit of enhanced stability against protease degradation.[15] Efforts to prepare basic aza-amino acid residues have typically involved building block synthesis in solution,[16−18] which restricts analog generation. Recently, our laboratory reported the synthesis of aza-Lys peptides by a route featuring the copper-catalyzed addition of Mannich reagents to an aza-propargylglycine residue.[19] This so-called A^3 reaction[20] was amenable to solid phase to provide rigid lysine analogs of type C (Figure 1) possessing a variety of tertiary amines on the side chain. Moreover, hydrogenation of the triple bond gave access to the *Z*-olefin analogs D. In principle, further reduction of the triple bond may give access to aza-lysine analogs of type B. The A^3-route does however have limitations in requiring typically secondary amines, a four-carbon side chain length, and extra steps to prepare saturated analogs.

In our efforts to make constrained *N*-aminoimidazolidin-2-one peptides,[21] we have recently explored the application of 1,2-dibromoethane in the alkylation of both urea nitrogen of a

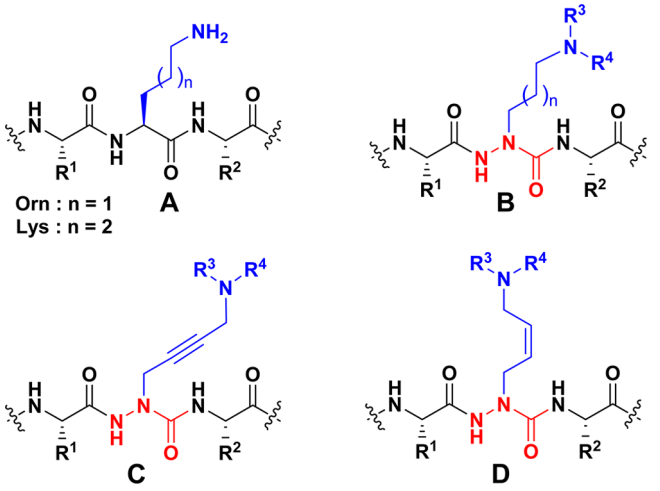


Figure 1. (A) Ornithine and lysine in native peptide, and aza-lysine analogs possessing (B) saturated (C) alkyne and (D) *Z*-olefin side chains.

semicarbazone to prepare the heterocycle. Considering the rate of nitrogen alkylation, we perceived that the more acidic semicarbazone nitrogen could be alkylated with an α-bromo-ω-chloro alkane to furnish an alkyl chloride side chain. Subsequent displacement of the chloride using a variety of amines may then provide access to a diverse array of aza-residues with basic side chains. This strategy has now been realized using

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both solution- and solid-phase methods, which have provided a wide diversity of peptide analogs possessing basic aza-residues.

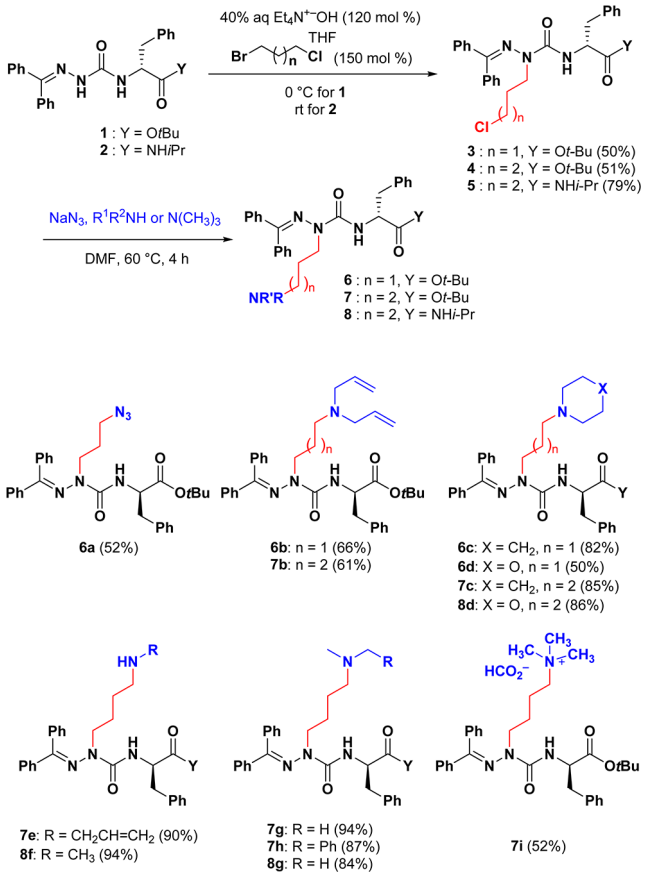
Aza-lysine analogs were pursued initially by a solution-phase method (Scheme 1) featuring alkylation of benzhydrylidene-azaGly-Phe-O*t*Bu (**1**) and benzhydrylidene-azaGly-Phe-N*i*Pr (**2**).[22] Employing tetraethylammonium hydroxide (120 mol %, as a 40% aqueous solution) in THF, ester **1** and amide **2** were alkylated with 1-bromo-3-chloropropane and 1-bromo-4-chlorobutane to provide the chloroalkyl semicarbazones **3**−**5**, which were converted into aza-Orn and aza-Lys dipeptides by chloride displacement with different amines. Racemization of esters **3** and **4** (up to 15%) was however detected from the alkylation of *tert*-butyl ester **1** at room temperature, but was avoided by decreasing the temperature to 0 °C. On the other hand, no loss of configuration was observed using amide **2** even at rt. Chloroalkyl semicarbazones **3**−**5** were then converted to analogs of aza-Orn and aza-Lys **6**−**8** from reactions with sodium azide, primary and secondary amines, and trimethylamine in DMF at 60 °C (Scheme 1). The relatively polar final dipeptides were purified by preparative HPLC on a C18 column, except for compounds **6a** and **6d**, which were purified by flash chromatography on silica gel.

A method for the synthesis of azapeptides possessing basic aza-amino acid residues was next developed on solid phase. Growth Hormone Releasing Peptide-6 (GHRP-6, His-D-Trp-Ala-Trp-D-Phe-Lys-NH_2), a cluster of differentiation 36 receptor (CD36) ligand,[23] was chosen for this study, because of its interesting biological activity and challenging chemical structure. In particular, [azaLys]-GHRP-6 analogues were targeted, because of the presence of two lysine residues in the related peptide EP80317 (Haic-2Me-Trp-D-Lys-Trp-D-Phe-Lys-NH_2), which has exhibited CD36 selectivity.[24,25] Azapeptide analogs of GHRP-6 were thus targeted possessing respectively basic aza-residues at the 3-, 4-, and 6-positions.

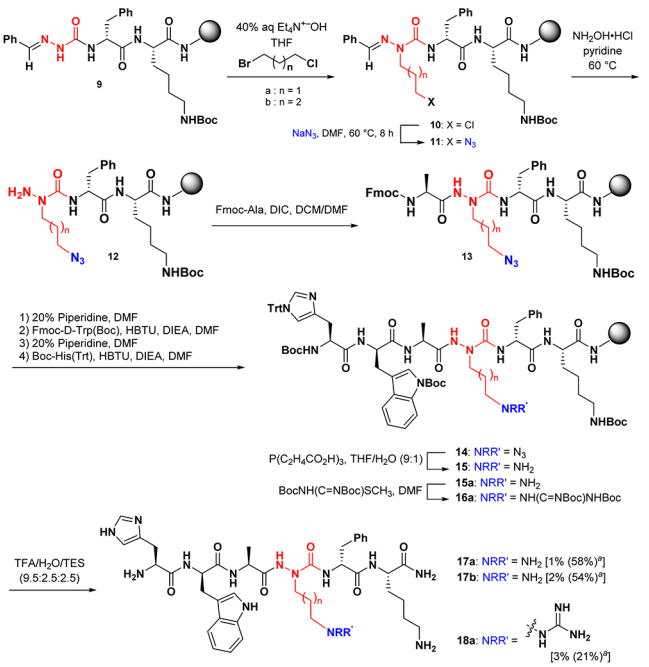
Initially, the set of [azaOrn^4]-, [azaLys^4]-, and [azaArg^4]-GHRP-6 analogs was pursued in order to demonstrate a solid-phase protocol for preparing aza-derivatives of the common naturally occurring basic amino acids. On rink amide resin, benzylidene-azaGly-D-Phe-Lys(Boc) **9** (Scheme 2) was synthesized as previously described.[26] Alkylation of resin **9** was performed with 1-bromo-3-chloropropane and 1-bromo-4-chlorobutane, respectively, at room temperature using tetraethylammonium hydroxide (200 mol % of a 40% aqueous solution) in THF to provide the chloroalkyl resins **10a** and **10b**. Conversion was assessed to be complete by treating aliquots of resin **10** with TFA/H_2O/TES (9.5:0.25:0.25) and examination of the residue after resin filtration by LC-MS. Resins **10** were respectively treated with sodium azide in DMF to provide the corresponding azides **11a** and **11b**. The semicarbazone was removed using a 1.5 M solution of hydroxylamine hydrochloride in pyridine, and the resulting semicarbazides **12** were coupled to Fmoc-Ala by way of its symmetric anhydride using diisopropylcarbodiimide (DIC) to provide azatetrapeptides **13**.[26] Elongation of the sequence was then performed using conventional Fmoc-based solid-phase peptide synthesis (SPPS).[27]

Azides **14** were chemoselectively reduced with tris(2-carboxy)ethylphosphine (TCEP) to provide the corresponding amines **15**.[28,29] Resin cleavage was then performed using a cocktail of TFA/H_2O/TES (9.5:0.25:0.25) to provide respectively [azaOrn^4]- and [azaLys^4]-GHRP-6 (**17a** and **17b**) in 58% and 54% crude purities. Purification of the crude azapeptides by

Scheme 1. Solution-Phase Synthesis of Aza-Lys and Aza-Orn Dipeptides



Scheme 2. Synthesis of [AzaLys^4]-, [AzaOrn^4]-, and [AzaArg^4]-GHRP-6 Analogs



[a]Isolated yield calculated from resin loading and crude purity ascertained by LC-MS.

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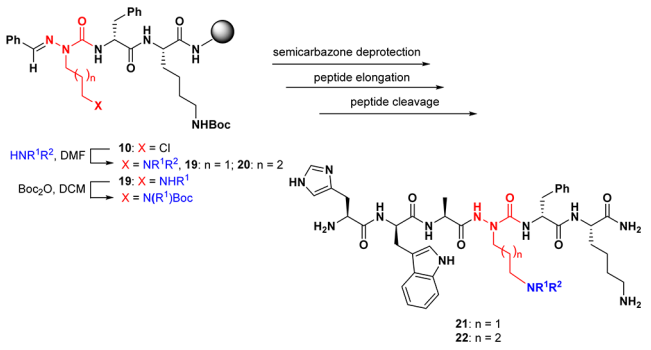
HPLC on a C18-column gave 1% and 2% overall yields of **17a** and **17b**. Alternatively, amine **15a** was treated with *N*,*N*-bis(Boc)-*S*-methylisothiourea in DMF at rt for 12 h to provide [azaArg^4]-GHRP-6 resin **16a**,[30] which was cleaved from the resin using the same cocktail to provide [azaArg^4]-GHRP-6 (**18a**) in 21% crude purity, and 3% overall yield after purification by HPLC.

Chloroalkyl resins **10** were subsequently employed to prepare a library of diverse [azaLys]-GHRP-6 derivatives (Scheme 3).

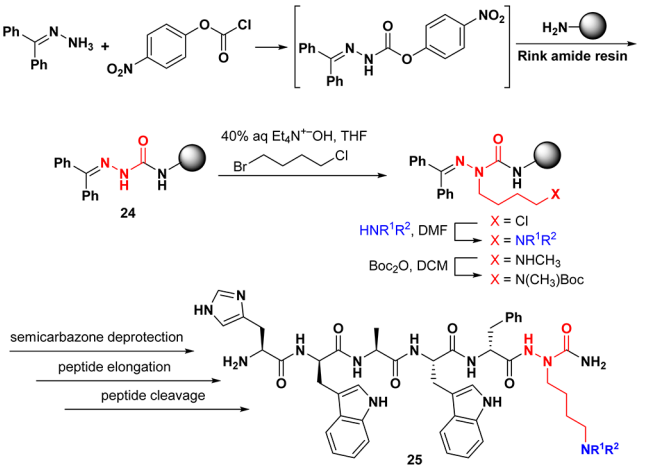
Scheme 3. Synthesis of [AzaLys^4]-GHRP-6 Derivatives



Employing various primary and secondary amines to displace chlorides **10a** and **10b** in DMF, substituted azaOrn and azaLys resins **19** and **20** were respectively synthesized. In the case of secondary amine analogs, the amine was subsequently protected as the corresponding *N*-Boc derivative by treating the resin with di-*tert*-butyldicarbonate in dichloromethane. The semicarbazone protecting group was removed, with the peptides elongated and cleaved from resin as previously described above. Final [azaOrn^4] and [azaLys^4]-GHRP-6 analogs **21** and **22** were obtained in 23–63% crude purities and isolated in 2–9% overall yields after purification by HPLC (Table 1; see Supporting Information for details). Employing a similar synthetic strategy [azaLys^3]-GHRP-6 analogues **23** were prepared from benzylidene-azaGly-Trp(Boc)-D-Phe-Lys(Boc) rink amide resin (Table 1).[31]

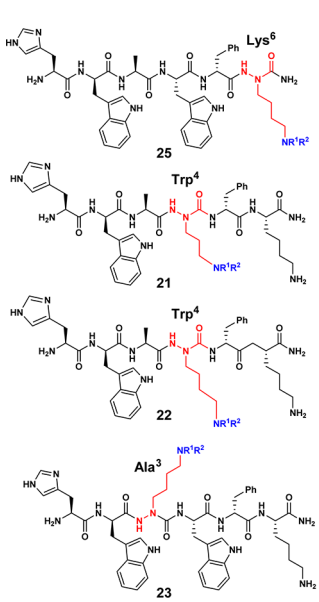
Finally, [azaLys^6]-GHRP-6 analogs **25** were prepared using methyl-, dimethyl-, and trimethylamines (Scheme 4). Benzylidene-azaGly rink amide resin **24** was obtained by removal of the Fmoc protection from rink amide resin, and treatment of the amine resin with 4-nitrophenyl 2-(diphenylmethylidene)-carbazate, which was prepared from the reaction of benzophenone hydrazone with *p*-nitrophenylchloroformate (Scheme 4). LCMS analysis of a cleaved resin aliquot of **24** showed 98% conversion to the *N*-(diphenylmethylidene)glycinamide product possessing the desired molecular ion. To introduce the side chains, resin **24** was alkylated with 1-bromo-4-chlorobutane and tetraethylammonium hydroxide (120 mol %, as a 40% aqueous solution) in THF, followed by chloride conversion into an amine. In the case of the methylamine analog, the amine was subsequently protected as the corresponding *N*-Boc derivative before peptide elongation. After removal of the semicarbazone group, azapeptides were elongated and cleaved from the resin as previously described.

Scheme 4. Synthesis of [azaLys^6]-GHRP-6 Analogues



In summary, 16 new GHRP-6 analogs were synthesized possessing aza-residues with different basic amine side chains (Scheme 2 and Table 1). This method has provided access to azaLys, azaOrn, and azaArg peptide sequences from a common diversity-oriented strategy featuring alkylation of an azaGly residue with an α-bromo-ω-chloroalkane followed by displacement of the resulting chloride with various amines. With this set of GHRP-6 analogs possessing basic aza-residues in hand, their impact on CD36 affinity and activity is currently being examined and will be reported in due time. Considering the power of this method for preparing azapeptides possessing diverse basic aza-residues, this approach should find significant applications in the study of various events featuring the post-translational modification and activity of lysine and arginine containing peptide structures.

Table 1. [AzaLys]-, [AzaOrn]-GHRP-6 Derivatives



entry	NR^1R^2	% crude / isolated purity[a,b] (yield[c])	HRMS m/z calcd (m/z observed)
25a	NH_2	46 / 95 (7)	896.4291 (896.4287)
25f	$NHCH_3$	41 / 95 (4)	910.4447 (910.4465)
25g	$N(CH_3)_2$	39 / >95 (5)	902.0549 (902.0539)
25i	$N^+(CH_3)_3 \cdot HCO_2^-$	38 / >99 (7)	916.4941 (916.4939)
21b	$N(CH_2CH{=}CH_2)_2$	49 / >99 (2)	904.4917 (904.4910)
21c	N⟨⟩	23 / >99 (2)	870.5097 (870.5086)
21d	N⟨O⟩	23 / 95 (2)	870.4744 (870.4706)
21e	$NH(CH_2CH{=}CH_2)$	55 / >99 (2)	864.4694 (864.4857)
22b	$N(CH_2CH{=}CH_2)_2$	23 / >99 (2)	918.5073 (918.5 047)
22h	$N(CH_3)CH_2Ph$	58 / >99 (9)	920.5253 (920.5242)
22j	$NHCH_2CH_2OCH_3$	60 / >99 (2)	896.4467 (896.4859)
23a	NH_2	63 / 98 (1)	953.4869 (953.4870)
23c	N⟨⟩	41 / >99 (3)	1021.5495 (1021.5477)

[a]Crude purity ascertained by LCMS analysis at 214 nm using H_2O (0.1% FA)/MeOH (0.1% FA) and H_2O (0.1% FA)/MeCN (0.1% FA) as eluents. [b]Isolated purity ascertained in same manner as crude purity. [c]Isolated yields calculated from resin loading.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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